DOCUMENT RESUME

ED 482 949 SE 067 238

AUTHOR	Brantley, L. Reed, Sr.; Demanche, Edna L.; Klemm, E. Barbara; Kyselka, Will; Phillips, Edwin A.; Pottenger, Francis M.; Yamamoto, Karen N.; Young, Donald B.
TITLE	Chromatography.
ISBN	ISBN-0-937049-28-X
PUB DATE	2003-00-00
NOTE	41p.; Produced by Hawaii University, Curriculum Research and Development Group, Foundational Approaches in Science Teaching Project.
AVAILABLE FROM	Foundational Approaches in Science Teaching Project, Curriculum Research and Development Group, 1776 University Avenue, Honolulu, HI 96822-2463. e-mail: crdg@hawaii.edu; Web site: http://www.hawaii.edu/crdg.
PUB TYPE	Books (010) -- Guides - Classroom - Teacher (052)
EDRS PRICE	EDRS Price MF01 Plus Postage. PC Not Available from EDRS
DESCRIPTORS	*Chromatography; *Chemical Analysis; *Chemistry; Instructional Materials; Lesson Plans; *Science Activities; *Science Instruction; Secondary Education; Teaching Methods

ABSTRACT

This booklet presents some activities on chromatography. Directions for preparing leaf pigment extracts using alcohol are given, and paper chromatography and thin-layer chromatography are described as modifications of the basic principles of chromatography. (KHR)

Reproductions supplied by EDRS are the best that can be made
from the original document.

ED 482 949

Chromatography

PERMISSION TO REPRODUCE AND DISSEMINATE THIS MATERIAL IN MICROFICHE ONLY HAS BEEN GRANTED BY

D. Young

TO THE EDUCATIONAL RESOURCES INFORMATION CENTER (ERIC)

2B

U.S. DEPARTMENT OF EDUCATION
Office of Educational Research and Improvement
EDUCATIONAL RESOURCES INFORMATION CENTER (ERIC)

☒ This document has been reproduced as received from the person or organization originating it.

☐ Minor changes have been made to improve reproduction quality.

• Points of view or opinions stated in this document do not necessarily represent official OERI position or policy.

Foundational Approaches in Science Teaching
Curriculum Research & Development Group

BEST COPY AVAILABLE

Chromatography

Foundational Approaches in Science Teaching
Curriculum Research & Development Group

3

FAST Principal Authors

L. Reed Brantley, Sr. Edna L. Demanche, E. Barbara Klemm, Will Kyselka, Edwin A. Phillips, Francis M. Pottenger III, Karen N. Yamamoto, and Donald B. Young

FAST Contributors

Harriet Ajimine, Leslie R. Allen, Robert Campbell, Ronald M. Flegal, Judith Hayashida, Arthur R. King, Jr., Harold J. Morowitz, Janet Ohta, Larma Pottenger, Martin W. Schein, Barbara Siegel, Sanford M. Siegel, Patricia O'Sullivan Robins, Caren V. Walsh, and John A. Winnie

Curriculum Research & Development Group Staff

Arthur R. King, Jr., Director
Francis M. Pottenger III, Director, Science Section
Francis M. Pottenger III and Donald B. Young, Directors, *FAST* Project
Edith K. Kleinjans, Managing Editor
Gayle Y. Hamasaki, Publications Coordinator

Published by the Curriculum Research & Development Group
©1987 by the University of Hawai'i
Reprinted 2003

All rights reserved

Distributed by the *FAST* Project
Curriculum Research & Development Group
1776 University Avenue
Honolulu, Hawai'i 96822-2463

E-mail: crdg@hawaii.edu
On the web: http://www.hawaii.edu/crdg

ISBN 0-937049-28-X
ISBN 0-937049-27-1 (set)

Contents

Introduction to Chromatography. 1
Preparing Leaf Pigment Extracts with Alcohol. 3
Concentrating Leaf Pigment Extract 6
Paper Chromatography 9
1. Preparing Chromatographic Paper Strips. 10
2. Preparing Separation Chambers 12
3. Spotting Samples on Chromatographic Paper 13
4. Developing Paper Chromatograms 14
Thin-Layer Chromatography. 18
1. Preparing Coated Vials. 19
2. Preparing Separation Chambers 23
3. Spotting Samples on Silica Gel. 24
4. Developing Thin-Layer Chromatograms 26
Special Problems. 30
Glossary of Chromatographic Terms 33
References 35

Introduction to Chromatography

In 1903 a Russian named M. Tswett invented a technique called *chromatography* for separating and identifying the components of a mixture. In the 1930s scientists throughout the world became interested in this technique. They expanded Tswett's technique to include examining many kinds of solutions and mixtures. Modern scientists now use chromatography extensively.

The basis of all chromatographic techniques is that different substances in a mixture have (1) different solubilities and (2) different abilities to be absorbed and adsorbed by certain materials. A sample of a mixture to be separated is placed on an absorbent material called a *sorbent.* The sorbent with the sample of mixture on it is put into a chamber containing a small quantity of liquid called the *solvent.* As the solvent moves up the absorbent material by capillary action, the components of the mixture separate. The result of the process is called a *chromatogram.* The chromatogram shows the components of the mixture deposited at different positions on the sorbent. The substances can then be identified by measuring the height to which each moved and comparing it with known standards.

An example of the use of the chromatography is shown in Figure 1. Samples of three known drugs and a sample of body fluid taken from a patient believed to have taken an overdose of drugs were placed on the sorbent on the left. The sorbent was placed in a chamber containing a solvent. The completed chromatogram is shown on the right. Notice that drugs contained in the unknown sample can now be identified as drugs A and B.

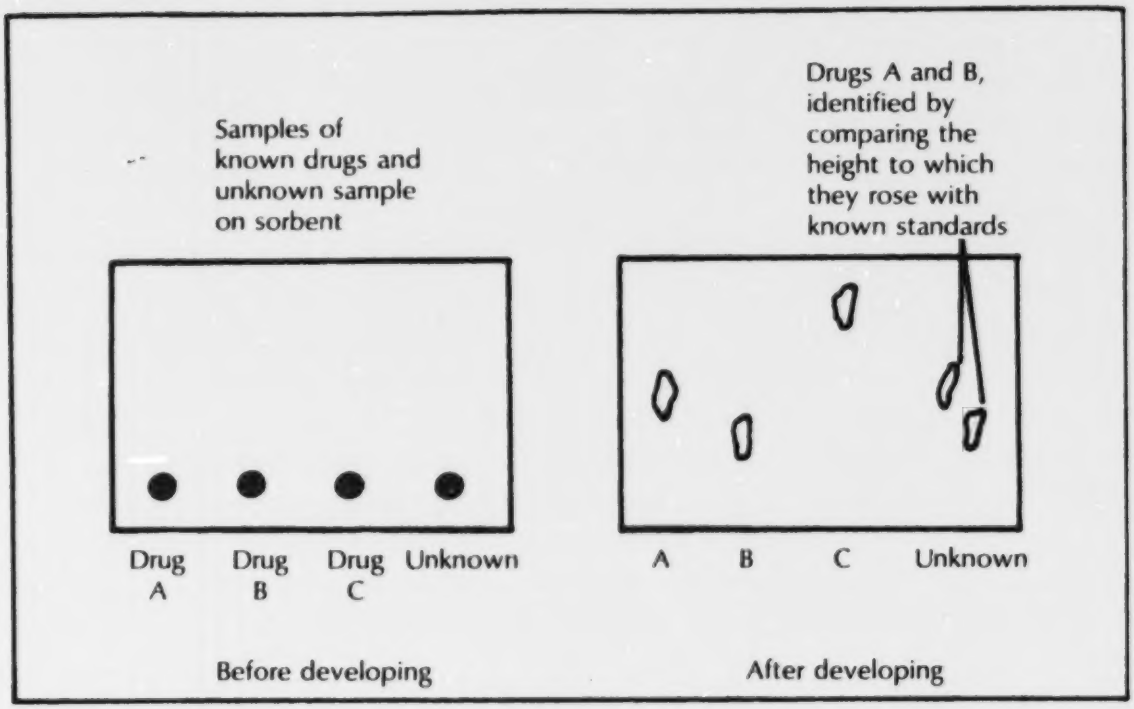


Figure 1 Example of the use of chromatography

There are many modifications of the basic principles of chromatography. Two methods, *paper chromatography* and *thin-layer chromatography*, are described in this booklet. Both methods can be used for separating and identifying plant pigments.

Before these methods are described, directions are given for preparing leaf pigment extracts using alcohol.

Preparing Leaf Pigment Extracts with Alcohol

Plant pigments are soluble in alcohol. In the technique described below, methyl alcohol is used to extract pigments from leaves.

CAUTION: Alcohol is flammable. If it catches fire, **smother the flames** by covering the container with a pan, a tray, or a plate. **Wear an apron and goggles.**

Materials

- two 250-ml Erlenmeyer flasks
- two 250-ml beakers
- two 600-ml beakers
- heat source
- 50 ml 95% methyl alcohol (ditto fluid)
- forceps
- test tube with stopper
- green leaves
- #6 one-hole stopper
- 6 cm glass tubing
- 30 cm rubber tubing
- ice

Directions

1. Set up the apparatus for making an alcohol extract.
   a. Set up a hot-water bath and an ice-water bath as shown in Figure 2.
   b. Put about 50 ml of alcohol into a 250-ml Erlenmeyer flask. Put the flask of alcohol into the water bath. Heat.
   c. Put about 200 ml of water into a 250-ml beaker and heat to boiling.

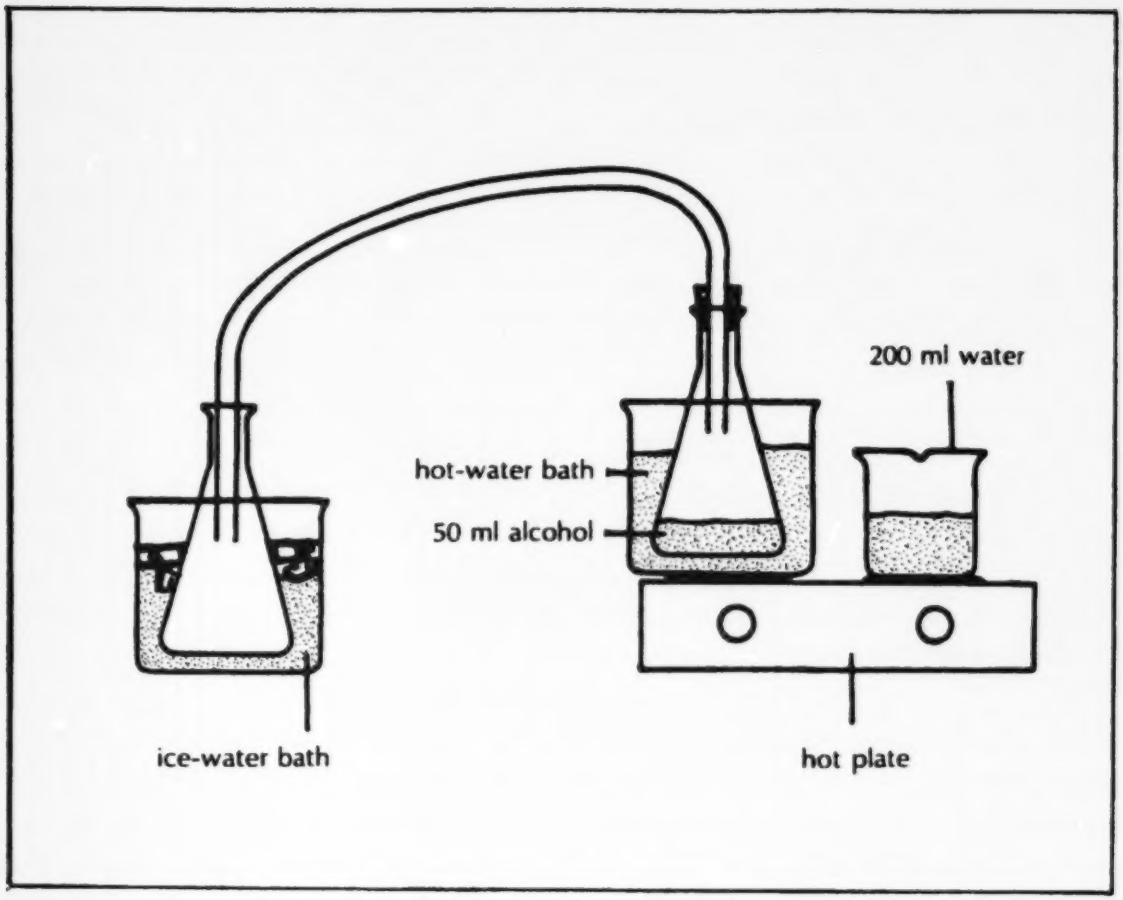


Figure 2 Apparatus for extracting leaf pigments

2. Dip one or two leaves from the same plant into the boiling water for about 1 minute. The hot water softens the plant tissues and makes it easier to remove the pigments from the leaves.
3. Remove the leaf from the boiling water. Put the leaf into the hot alcohol. After all the pigments are extracted, the leaf tissue appears white or almost colorless. Remove the leaf from the alcohol. Discard the leaf.
4. Allow the plant leaf pigment extract to cool. The extract should be clear, with no solid particles in it. If the extract is cloudy, filter it as shown in Figure 3.

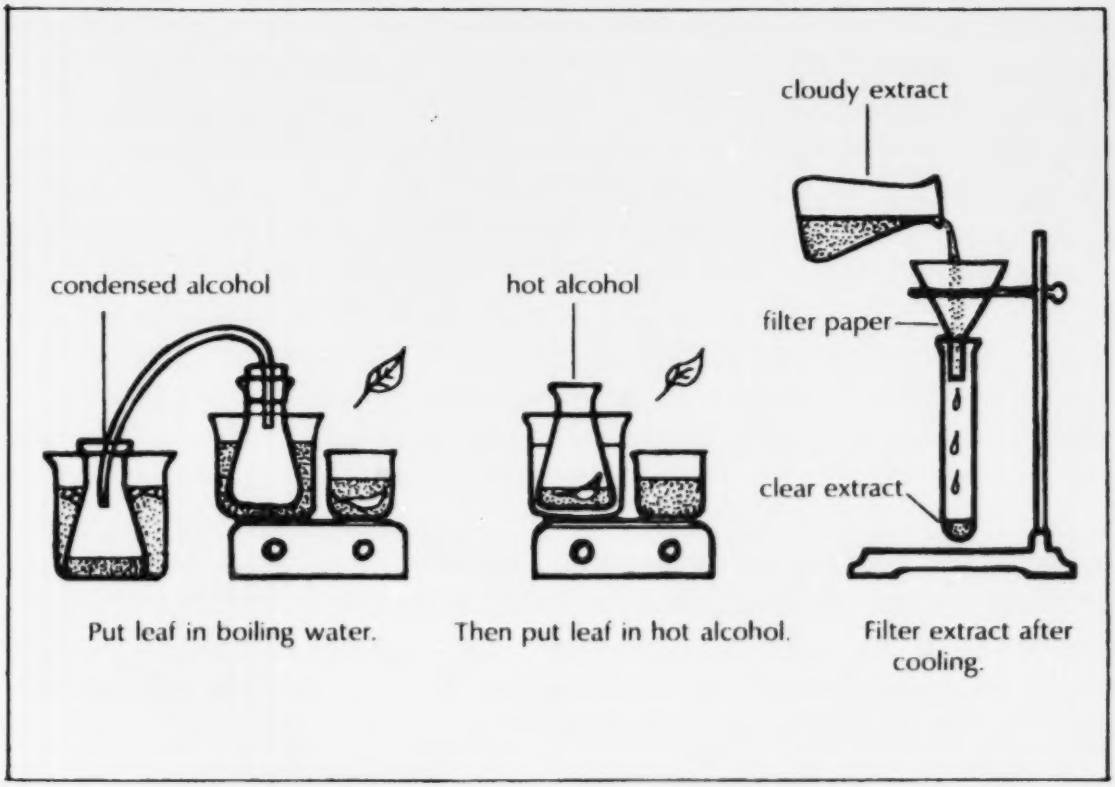


Figure 3 Procedure for extracting leaf pigments

5. Store the clear leaf pigment in a tightly stoppered test tube. Wrap the test tube in aluminum foil or some other material that keeps light out. Store the extract in a refrigerator if possible. Warm temperatures and especially light cause the pigments to decompose.
6. Label the test tube with
   - your name
   - the date
   - the type of leaves or other plant part

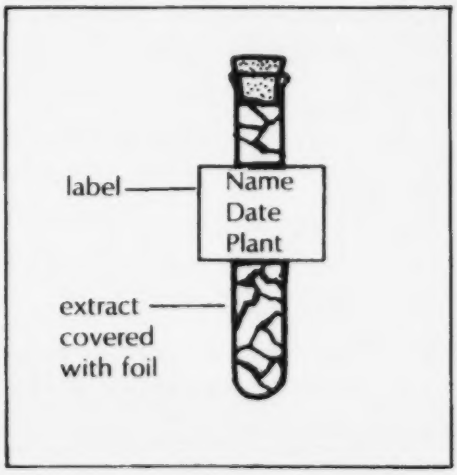


Figure 4 Labeled container of leaf pigment extract

Concentrating Leaf Pigment Extract

The leaf pigment extract contains the plant pigments that can be separated and identified by chromatography. If the leaf pigment extract is too dilute for use in chromatography, it must be concentrated.

Leaf pigment extract is concentrated by evaporating off most of the alcohol. Because of the danger of alcohol fires in concentrating the plant pigment extract, your teacher may have already prepared the concentrated extract.

CAUTION: **Alcohol vapors are highly flammable.** If possible, use a hot plate as the heat source. Keep a pan, dish, or tray handy for smothering any alcohol fires. Keep room well ventilated at all times. **Wear an apron and goggles.**

Materials

- two 250-ml beakers
- forceps
- capillary pipette
- heat source
- test tube containing plant pigment extract
- one-hole stopper to fit test tube
- ice
- 30 cm rubber tubing
- 5 ml ethyl alcohol

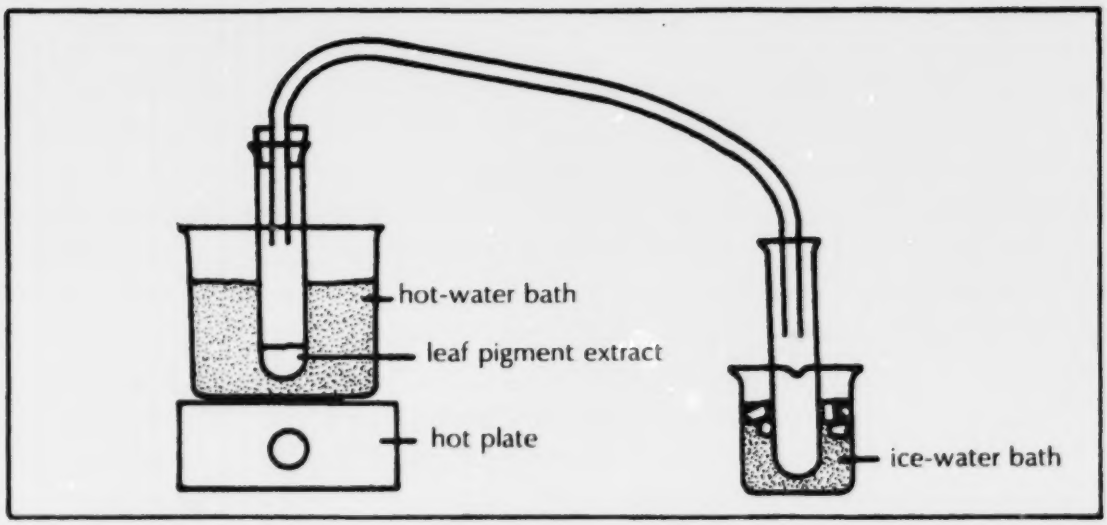


Figure 5 Apparatus for concentrating extracts

Directions

1. Remove the aluminum foil and stopper from the test tubes of plant pigment extract.
2. Set up the apparatus for concentrating the leaf pigment extract as shown in Figure 5. Heat.
3. After the alcohol is all, or nearly all, evaporated, the test tube will contain
   a. some sticky, dark-colored clumps of pigment clinging to the bottom and sides of the glass and
   b. probably a few drops of cloudy water

   With a pipette, draw off and discard the water, leaving a "dry" test tube with clumps of pigment in it.

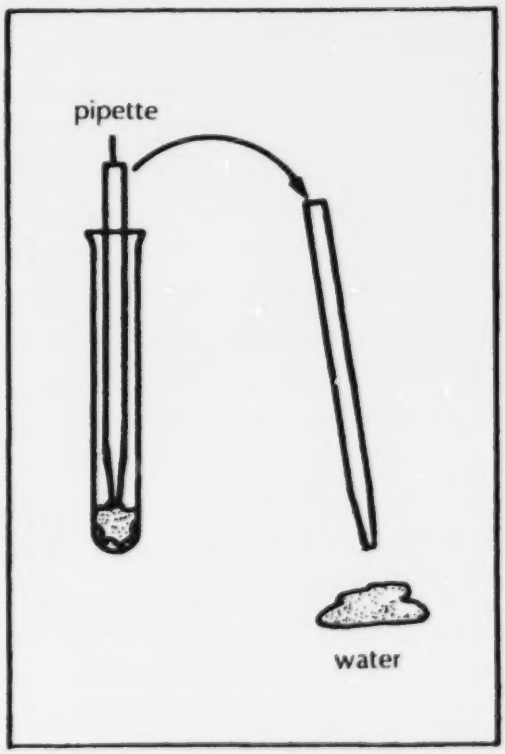


Figure 6 Removing water from extract

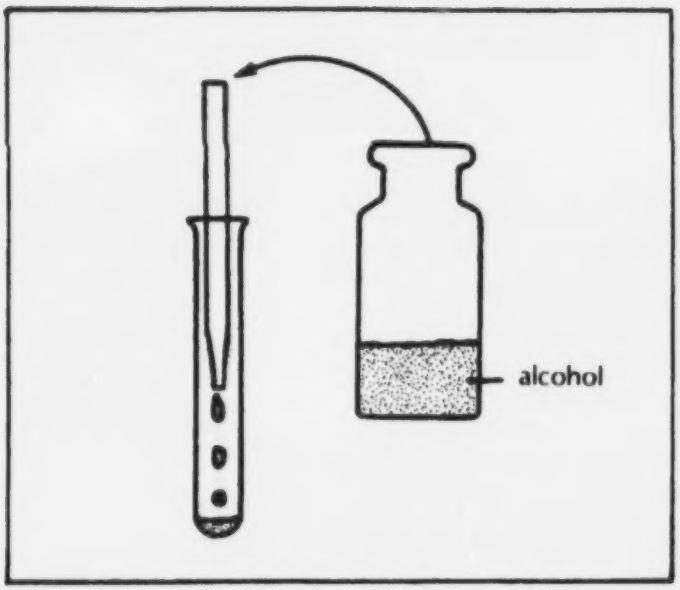


Figure 7 Redissolving the pigments in alcohol

4. Add four or five drops (no more) of ethyl alcohol to the test tube. This will redissolve the pigment into a clear, dark-colored, concentrated pigment extract ready for use in chromatography.
5. Stopper the test tube of plant pigment extract. Wrap the test tube in aluminum foil and label it.

   Keep the extract in a refrigerator, if possible, until you are ready to use it in chromatography.

Paper Chromatography

In *paper chromatography* (PC), filter paper or *chromatographic paper* is used as the sorbent. A *sorbent* is a solid material that absorbs and adsorbs substances. See Glossary at end of this booklet for definitions of these terms.

A sample of a mixture of substances to be separated and identified is put on a strip of chromatographic paper. The paper strip is placed in an airtight container called a *separation chamber*, which contains the *solvent*. A solvent is a liquid or a mixture of liquids used in chromatography to separate substances.

The solvent moves up the paper by capillary action. As it moves, the components of the mixture are carried along in the solvent. The substances in the mixture separate because of their slightly different solubilities in the solvent and their slightly different capacities for being absorbed and adsorbed by the sorbent.

On the finished *chromatogram*, the components of the mixture are deposited at different positions on the sorbent. Thus separated, the components can be identified by comparing the height to which each one moves with the heights to which known standards move. Figure 8 shows the chromatogram in the separation chamber. Figure 9 shows what the chromatogram might look like after the substances are separated.

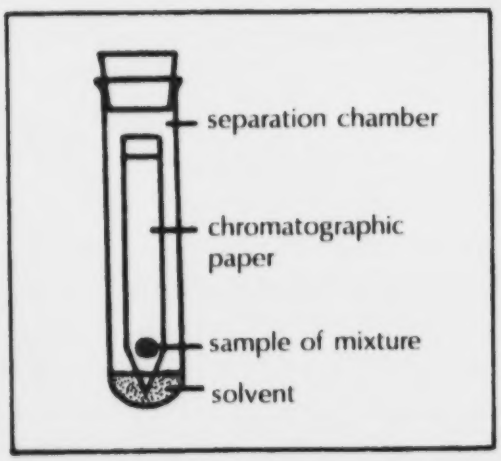


Figure 8 Apparatus for paper chromatography

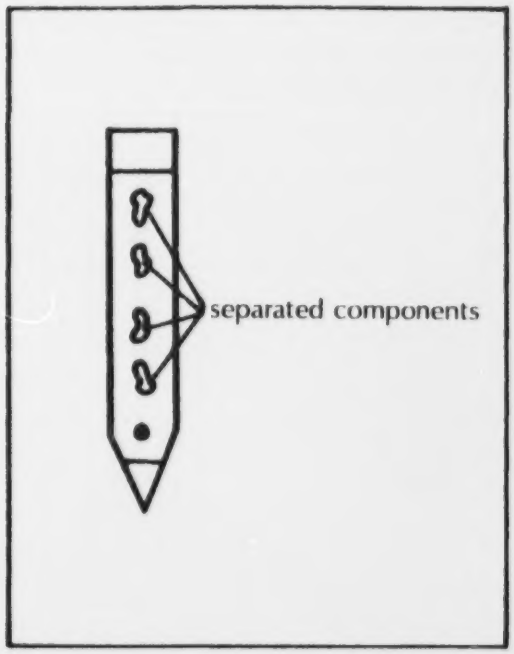


Figure 9 Example of a completed paper chromatogram

The following pages tell how to prepare materials for paper chromatography and how to develop paper chromatograms.

1. Preparing chromatographic paper strips

The paper strips for use in paper chromatography described in this booklet are cut from Whatman filter paper #1.

NOTE: Skin oils and moisture left by fingerprints prevent the even development of the chromatogram. Work with very clean hands and handle the paper by the edges whenever possible. Cutting the paper with a paper cutter reduces the possibility of soiling the paper.

Materials

- chromatography paper (Whatman filter paper #1)
- scissors and/or paper cutter
- sharpened lead pencil

Directions

1. Cut the chromatography paper into strips the size and shape of the pattern in Figure 10.
2. Draw a line across the tip of the paper as shown in the diagram. This *solvent line* shows the level the solvent should come to when the paper is lowered into the separation chamber.
3. Draw another line across the top of the paper as shown in Figure 10. This is the *finish line.* When the solvent reaches this line, the chromatogram can be removed from the separation chamber.
4. Wrap the papers in a clean material and store them in a clean, dry place.

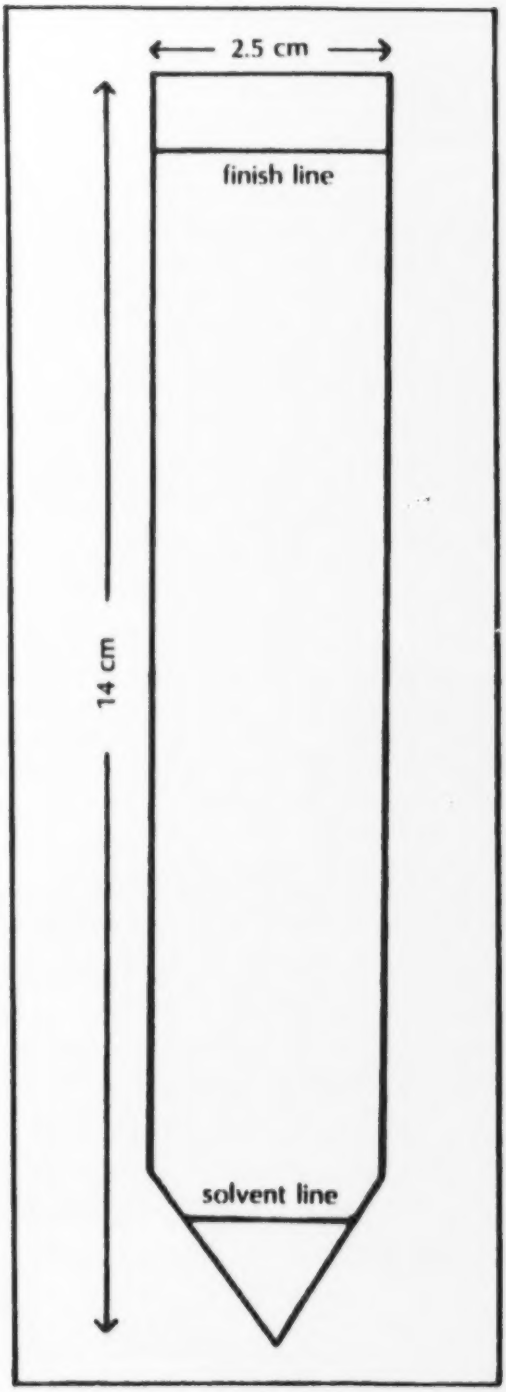


Figure 10 Pattern for cutting chromatographic strips

2. Preparing separation chambers

A *separation chamber* is an airtight test tube or jar containing the solvent used in developing a chromatogram.

Materials

- 1 culture tube, or any similar long, narrow glass tube with a tight cover (such as a tall, narrow olive bottle)
- solvent for ink (water)
- solvent for plant pigments (9:1 mixture of petroleum ether and ethyl alcohol)

CAUTION: Petroleum ether and ethyl alcohol are both poisonous, flammable liquids. **Do not use them near any flame. Wear an apron and goggles.**

Directions

1. Obtain a container with a tight cover to use as a separation chamber. The container must be tall enough to hold the chromatographic paper strip.
2. Pour about 4 ml of the solvent into the separation chamber.

   For ink spots, the solvent is water.

   For plant pigment extracts, the solvent is a 9:1 mixture of petroleum ether and ethyl alcohol.
3. Cover the chamber and let it stand for about 10 minutes to give time for the air in the tube to become saturated with the solvent vapors. This is very important for the best development of the chromatogram.

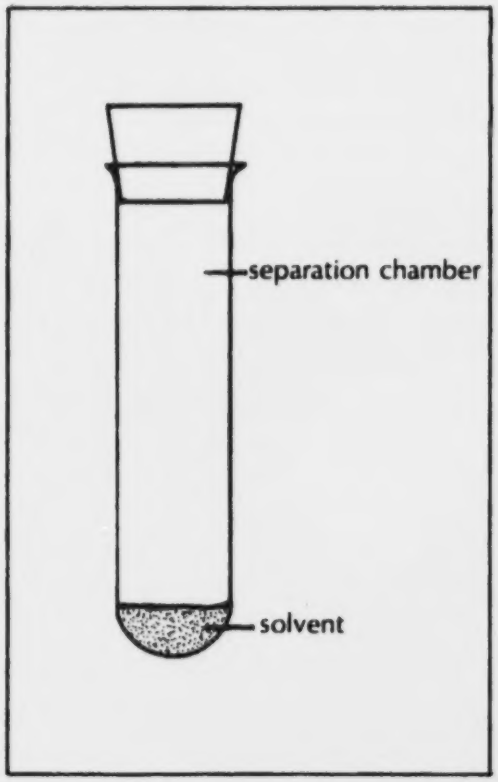


Figure 11 Separation chamber

3. Spotting samples on chromatographic paper

For good chromatographic separation, spots of the mixture on the paper must be small but concentrated. Placing samples of mixtures on the chromatographic paper to be separated is called *spotting.*

Materials

- sheet of soft, absorbent paper toweling
- 2 capillary pipettes
- washable black ink in a test tube (Keep the test tube stoppered when not in use.)
- small beaker, jar, or test-tube rack to hold the test tube of ink
- several strips of chromatographic paper
- sample of concentrated plant pigment extract

Directions

1. Practice spotting with washable black ink on a paper towel.
   a. Lay a folded paper towel on a clean surface.
   b. Dip a capillary pipette into the washable black ink. Control the flow of liquid into and out of the capillary pipette by placing your index finger over the top opening.
   c. Touch the pipette lightly to the paper towel. While waiting for the spot to dry, touch another spot.
   d. As soon as a spot is dry, touch it again to put another layer of ink on top of the first

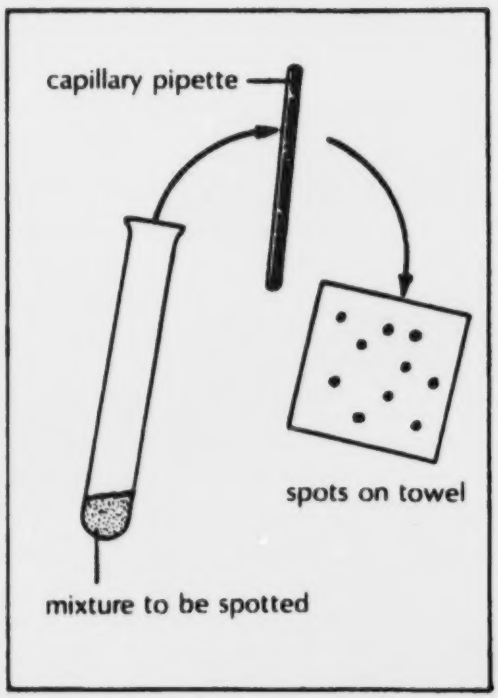


Figure 12 Spotting on a paper towel

one until all the spots are **small** and **dark** with several coats of ink.

2. As soon as you become skillful in making small, dark spots, spot ink on a clean strip of chromatographic paper about 0.5 cm above the solvent line. This spot is called the *origin.*
3. Spot samples of the plant pigment extracts you are investigating on other pieces of chromatographic paper.

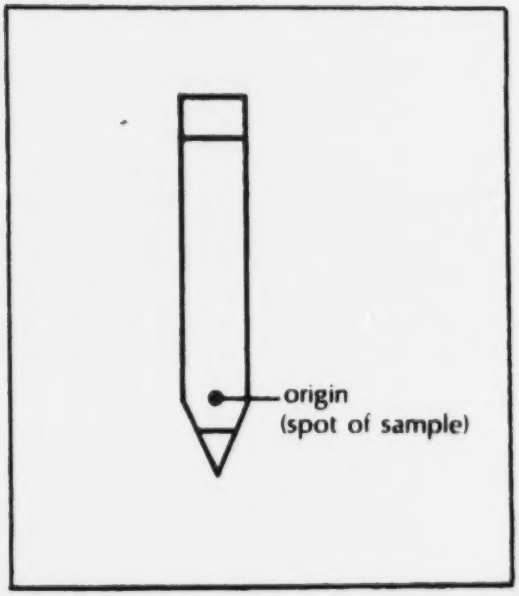


Figure 13 Spot on chromatographic paper

4. Developing paper chromatograms

To develop a paper chromatogram, the spotted paper strip is placed in a separation chamber containing a solvent. As the solvent is absorbed by the paper, the substances in the mixture separate by the rate at which they move up the paper strip.

NOTE: Keep finger marks off the paper strip because oils and moisture from the skin prevent good development. Keep the cap on the separation chamber at all times except to insert or remove the paper strip. Keep the paper dry.

Materials

- strip of chromatographic paper with an ink spot
- strip of chromatographic paper with a spot of concentrated plant pigment extract
- separation chamber containing solvent

- solvent for ink (water)
- solvent for plant pigments (9:1 mixture of petroleum ether and ethyl alcohol)

CAUTION: Petroleum ether and ethyl alcohol are both poisonous, flammable liquids. **Do not use them near any flame. Wear an apron and goggles.**

Directions

1. Develop the washable black-ink chromatograms.
   a. Predict what colored pigments will be found in the black ink.
   b. Put the solvent for ink chromatograms (water) into the chamber up to the solvent line.
   c. Put the paper strip with the ink sample into the separation chamber. Lower the paper carefully, making sure the solvent level in the chamber is no higher than the solvent line on the paper strip.
   d. Watch the solvent move up the paper. The leading edge of the solvent is called the *solvent front.*
   e. When the solvent front reaches the finish line at the top of the paper, the chromatogram is finished.

      Open the separation chamber and remove the chromatogram. Replace the cover on the separation chamber.

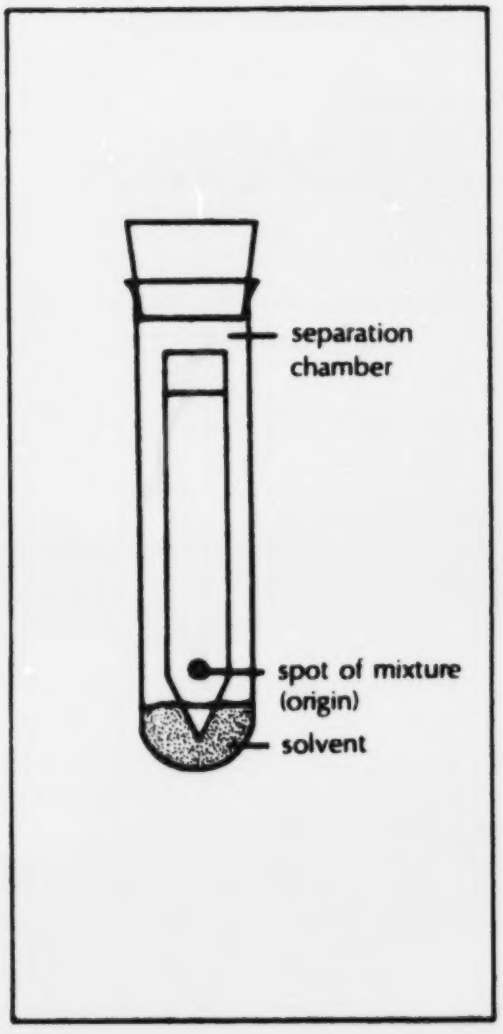


Figure 14 Developing a paper chromatogram

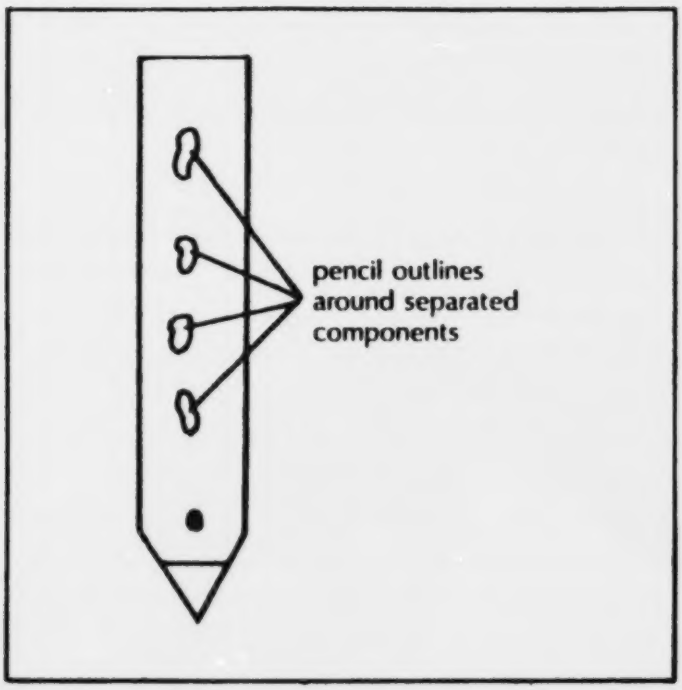


Figure 15 A finished chromatogram

f. With a pencil, outline each of the separated substances on the chromatogram. Write next to each spot what color it is. The colors may fade in a few hours, but your notes will remain.

g. Attach or copy the chromatogram onto a data table similar to Table 1. Fill in the data and keep the table in your notebook.

2. Develop the plant pigment chromatograms.

   a. Use a separation chamber containing a 9:1 mixture of petroleum ether and ethyl alcohol as the solvent.

   b. Except for the difference in the solvent used, follow the procedures for developing ink chromatograms.

Table 1 Sample Data Table for Paper Chromatograms

Sample _____

Age of sample _____

Sorbent _____

Solvent _____

Description of separated substances by color	Identification of substances

Comments

Name of researcher _____

Date _____

Either attach the paper chromatogram here with developed spots outlined in pencil or sketch the chromatogram.

Thin-Layer Chromatography

In *thin-layer chromatography* (TLC), silica gel, aluminum oxide, sugar, or some other thin, powdery substance is used as the *sorbent.* A sorbent is a solid material that absorbs and adsorbs substances. (See the Glossary in the back of this booklet for definitions of new terms.) The solid is mixed with a liquid to make a *slurry.* Usually a glass or metal sheet is dipped in the slurry to coat it with the sorbent. In the method described here, a glass vial is coated with the sorbent.

A sample of a mixture of substances to be separated and identified is put on the coated vial. The vial is placed in an airtight container called a *separation chamber,* which contains the *solvent.* A solvent is a liquid or a mixture of liquids that separates components of mixtures in chromatography.

The solvent moves up the sorbent coating on the glass vial by capillary action. As it moves, the components of the mixture are carried along in the solvent. The substances in the mixture separate because of their slightly different solubilities in the solvent and their slightly different capacities for being absorbed and adsorbed by the sorbent.

On the finished *chromatogram,* the components of the mixture are deposited at different positions on the sorbent. Thus separated, the components can be identified by comparing the height to which each one moves with the heights to which known standards move. Figure 16 shows the chromatogram in the separation chamber. Figure 17 shows what the chromatogram might look like after the substances are separated.

The following pages tell how to prepare materials for thin-layer chromatography and how to develop chromatograms.

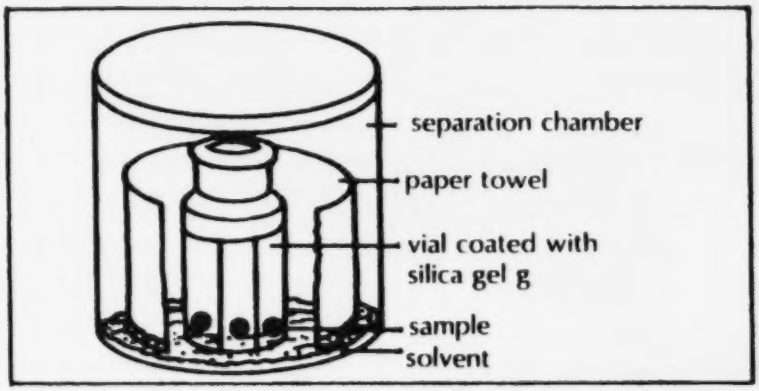


Figure 16 Apparatus for thin-layer chromatography

1. Preparing coated vials

The sorbent layer for thin-layer chromatography is prepared by coating vials with a finely powdered sorbent such as silica gel g, aluminum oxide, or sugar. The powdery sorbent is mixed in a liquid to form a *slurry*. In the procedure described below, the slurry is made by mixing silica gel g with water. The *g* indicates that a binder (plaster of Paris, calcium sulfate) has been added to the silica gel to make it stick to glass.

Materials (for each group of six students)

- 3 boards with six 4″ nails in each
- 15 to 20 vials
- small, capped bottle of alcohol
- graduated cylinder
- 15 g silica gel g
- scoopula or spoon
- widemouthed screw-cap jar about 10 cm tall (This is the shaking jar.)
- working area covered with clean newspaper

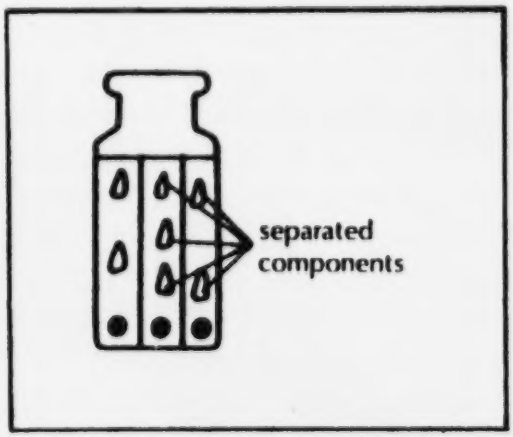


Figure 17 Example of a completed thin-layer chromatogram

Directions

1. Divide the class into teams of six persons each. Divide the following jobs among the team members. Directions for each job are given below.

Jobs	Materials Needed
Prepare the vials.	vials, alcohol, paper towel
Prepare the shaking jar.	shaking jar, paper towel, flame
Weigh the silica gel g.	silica gel g, balance, paper
Prepare the slurry.	15 g silica gel, shaking jar, graduated cylinder
Coat the vials.	slurry, clean vials, nail board
Dry and score the vials.	coated vials, oven, sharp pencil

The timing is important because once the silica gel is mixed with water, it hardens rapidly. Everything must be ready for immediate use.

2. Prepare the vials. Silica gel will not readily stick to vials with finger grease or dust on them.
   a. Wash and dry the vials.
   b. Dip a piece of paper towel in alcohol and "swipe" the outside of each vial to remove any traces of oil left on the vials.
   c. Place the vials on a nail board so they will not be touched accidentally.

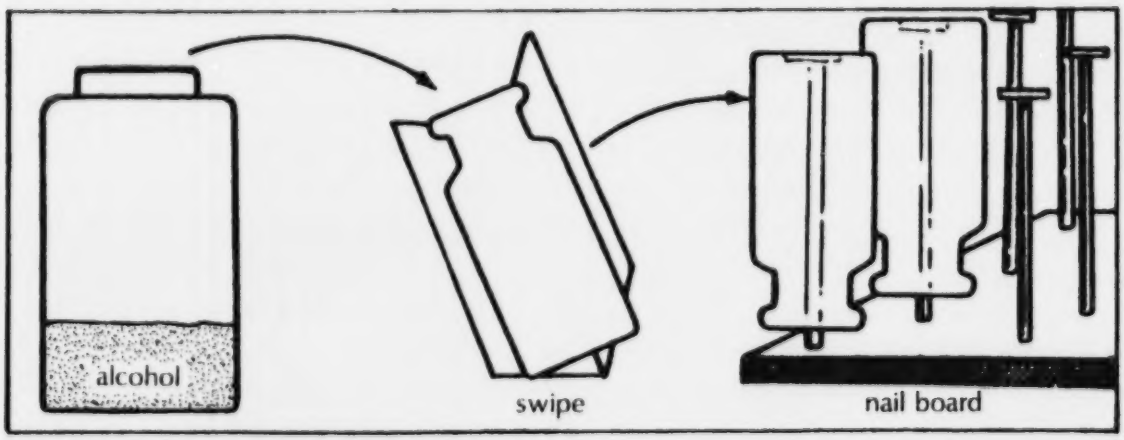


Figure 18 Swiping vials to remove grease

25

3. Prepare the shaking jar. It must be dry because moisture can cause the silica gel powder to cake and make lumps in the finished mixture.
   a. Wash the shaking jar. Dry it thoroughly over a warm flame or in an oven.
   b. Take the dry jar to the place where the silica gel is being weighed.
4. Weigh the silica gel g. *Silica gel g* is a very light powder that blows away in the slightest breeze. It is also expensive. Work in a place free of drafts from windows or from students passing by.
   a. Crease half a sheet of clean notebook paper and fold lengthwise.
   b. Weigh it.
   c. Weight out 15 grams of silica gel g. Do not count the weight of the paper.
   d. Transfer the powder into the dry shaking jar.
5. Prepare the slurry. A *slurry* is a solution of a liquid and a solid. In this case, the liquid is water and the solid is silica gel g.
   a. Measure 25 ml of water into a graduated cylinder.
   b. Add the water to the silica gel in the shaking jar and give the mixture one or two shakes. This causes pressure

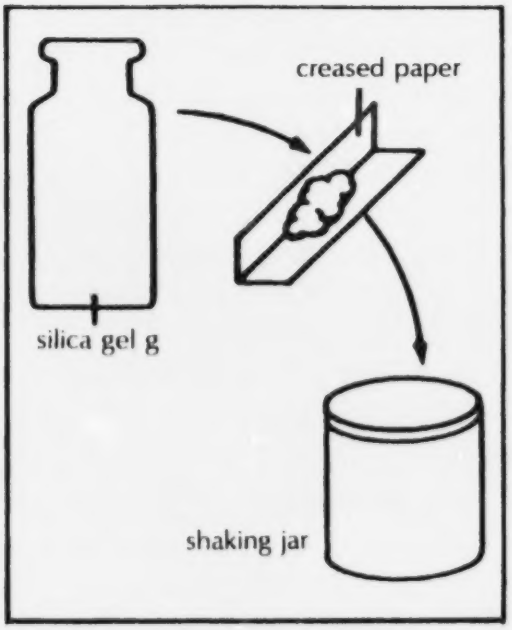


Figure 19 Putting dry silica gel into the shaking jar

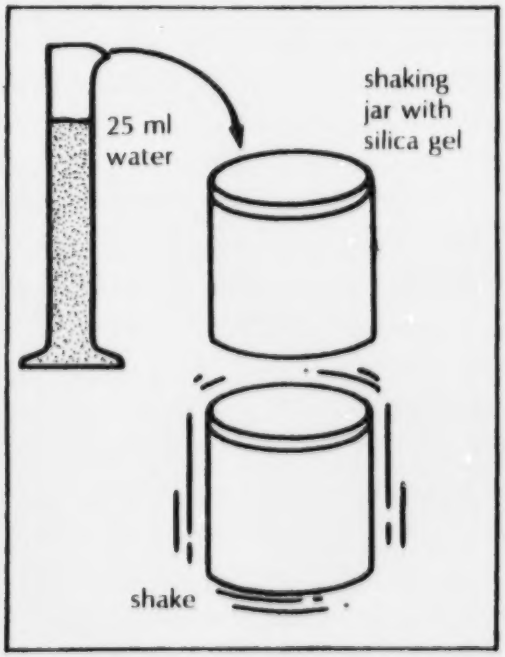


Figure 20 Preparing a slurry

to build up inside the jar. Unscrew the lid slightly to release the pressure.

c. Tighten the lid and shake vigorously for **1 minute**.

d. Examine the slurry to see if it is a thick but flowing mixture. If it is too thick, add a **few drops** of water. If it is too thin, keep shaking until it thickens.

6. Coat the vials as shown in Figure 21.

a. Hold a clean vial by its open end with one finger inside and the other fingers supporting the neck of the vial.

b. Tip the shaking jar with the slurry sideways and insert the vial.

c. Rotate the vial in the slurry until it is coated all around.

d. Lift it out of the jar and let excess slurry drip back into the jar.

e. Put the coated vial on the nail board to dry.

f. Repeat this procedure until all the slurry is used.

7. Dry and score the vials.

a. Either put the coated vials into a warm (110°C) oven for 20 to 30 minutes or let them dry at room temperature overnight.

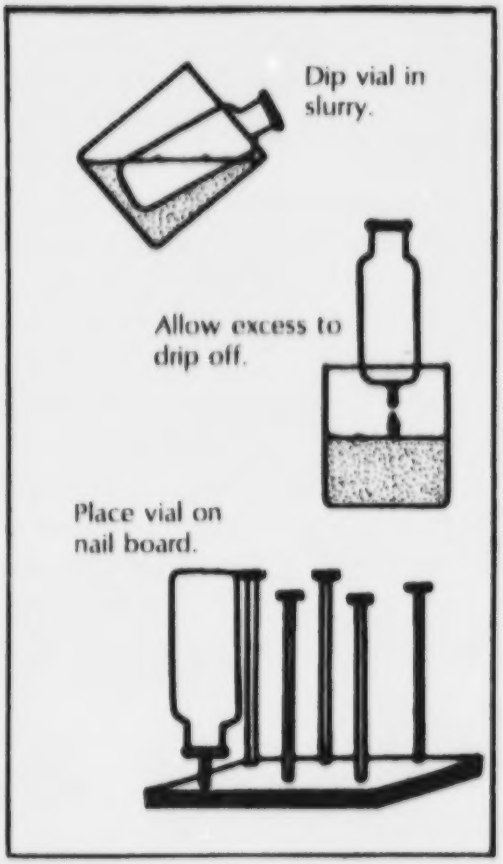


Figure 21 Coating the vials

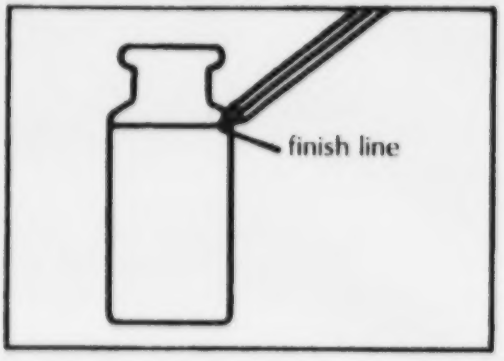


Figure 22 Coated vial ready for spotting

b. With a sharp pencil, draw a line (*score*) around the vial near the neck end. This marks the *finish line*. When the solvent reaches this line, the chromatogram can be removed from the separation chamber.

2. Preparing separation chambers

A separation chamber is an airtight jar containing the solvent used in developing a chromatogram.

Materials

- jar with a tight lid, about 12 cm tall—big enough to contain the coated vial and still be capped
- soft, absorbent paper toweling
- scissors
- solvent for ink (water)
- solvent for plant pigments (9:1 mixture of petroleum ether and ethyl alcohol)

CAUTION: Petroleum ether and ethyl alcohol are both poisonous, flammable liquids. **Do not use them near any flame. Wear an apron and goggles.**

Directions

1. Cut a sheet of paper toweling to line most of the inside of the jar. Leave a view slit at one side of the jar.
2. Pour a small amount of the solvent down the inner sides of the jar to wet the paper towel. Rotate the jar until the paper is entirely wet and clings smoothly to the sides of the jar. The purpose of the wet lining is to ensure a moist atmosphere in the jar.

   For ink spots, the solvent is water.

   For plant pigment extracts, the solvent is a 9:1 mixture of petroleum ether and ethyl alcohol.

3. Add enough solvent to the bottom of the jar to bring the liquid level to about 1 cm.
4. Cover the chamber and let it stand for a while before using. This gives time for the air in the chamber to become saturated with solvent vapors. This is very important for the best development of the chromatograms.

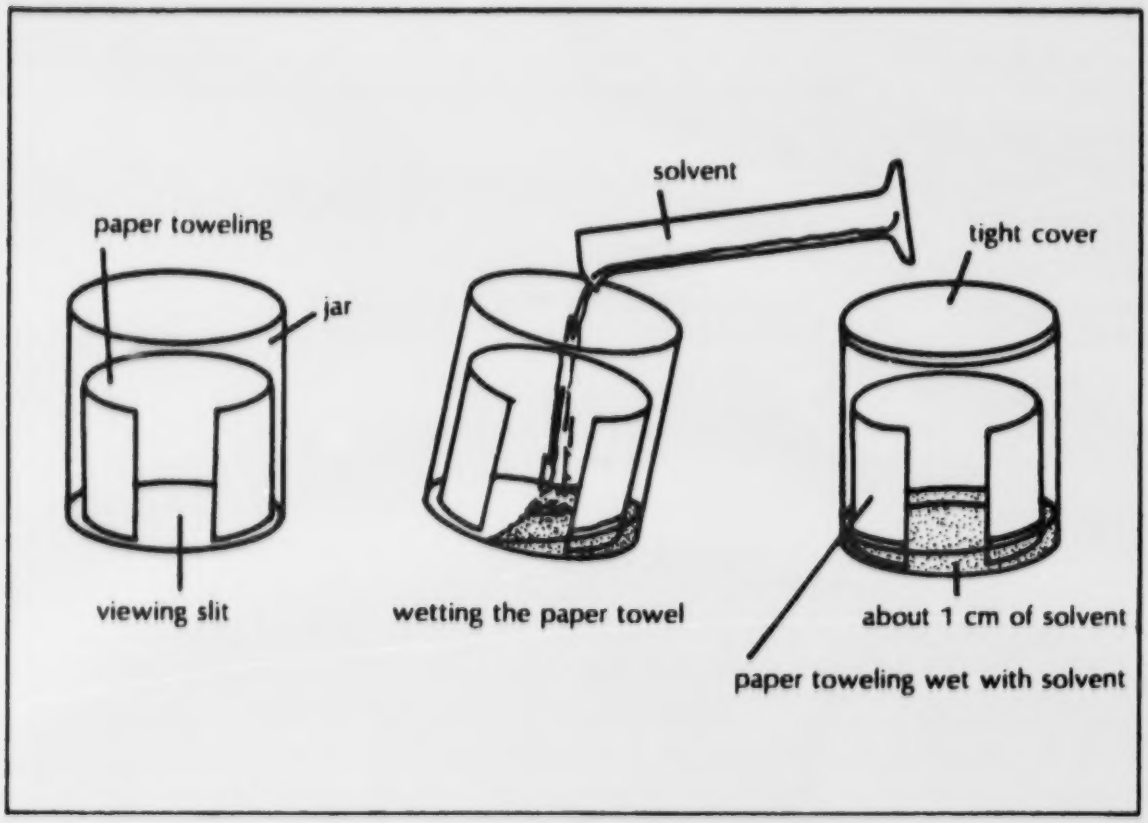


Figure 23 Preparing separation chambers

3. Spotting samples on silica gel

For good chromatographic separations, spots of the mixture on the silica gel must be small but concentrated. Placing samples of mixtures on the sorbent layer to be separated is called *spotting*.

Materials

- several sheets of soft, absorbent paper toweling
- 2 capillary pipettes
- washable black ink in a test tube (Keep the test tube stoppered when not in use.)
- a small beaker, jar, or test-tube rack to hold the test tube of ink
- several vials coated with silica gel g on a nail board.
- sample of concentrated plant pigment extract

Directions

1. Practice spotting on a paper towel as shown in Figure 24.
   a. Wrap a folded paper towel around your fist. Hold it in place with your thumb.
   b. Dip a capillary pipette into the washable black ink.
   c. Balance your wrists together to keep your hands steady and touch the pipette **lightly** to the towel. While waiting for the spot to dry, touch another spot.
   d. As soon as a spot is dry, touch it again to put another layer of ink on top of the first one. Try to make the spots **small** and **dark** with several coats of ink.

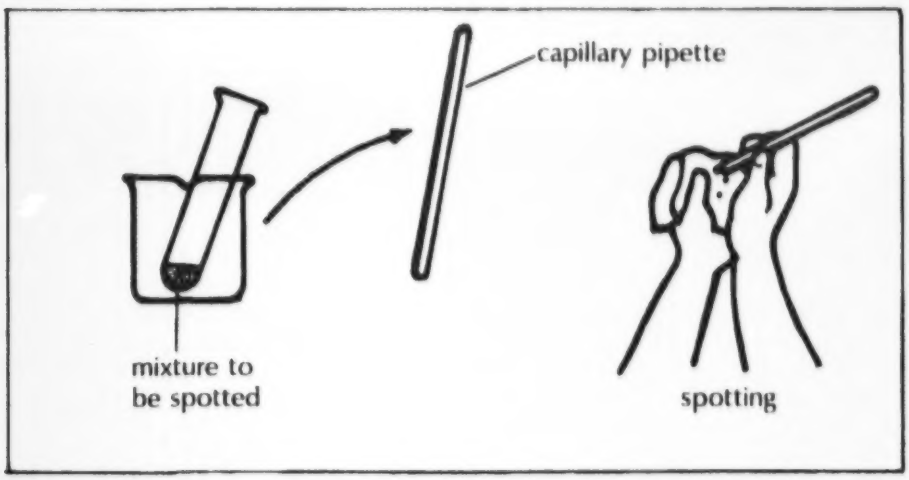


Figure 24 Spotting on paper towel

2. As soon as you become skillful in making small, dark spots, spot ink around a vial coated with silica gel. The spots should be about 1.5 cm from the bottom of the vial.
3. Score vertical lines with a sharp pencil point between the separated spots as shown in Figure 25. Scoring separates the developing chromatograms so that they do not mix with one another.

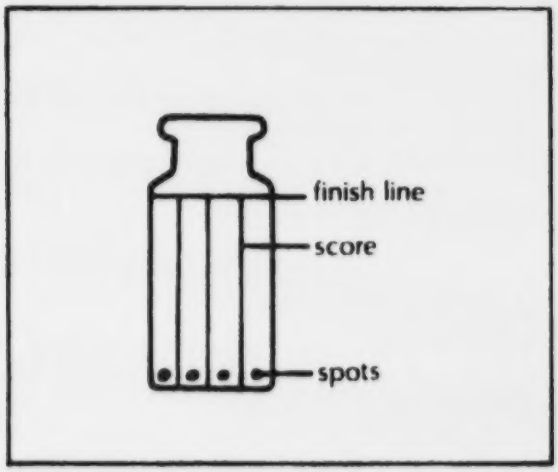


Figure 25 Spotted vial ready for separating

4. Spot samples of the plant pigment extracts you are investigating onto other coated vials.

4. Developing thin-layer chromatograms

To develop a thin-layer chromatogram, the spotted vial is placed in a separation chamber containing a solvent. As the solvent is absorbed by the sorbent layer, the substances in the mixture separate by the rate at which they move up the sorbent layer.

NOTE: Do not touch the silica gel coating on the vial. If the silica gel is scratched or flaked off, it will not make a chromatogram.

Materials

- nail board
- vial coated with silica gel g spotted with ink
- vial coated with silica gel g spotted with plant pigment extract
- separation chambers with appropriate solvents

Directions

1. Develop the washable black ink chromatogram.
   a. Predict what colored pigments will be found in the black ink.
   b. Pick up the vial at the neck end, inserting one finger inside the neck. If necessary, balance the vial with one finger against its bottom.
   c. Remove the lid from the separation chamber. Lower the vial into the separation chamber with its closed end down as shown in Figure 26. The ink spots should be just above the solvent.

      The solvent for ink chromatograms is water.
   d. Watch the solvent move up the silica gel coating. The leading edge of the solvent is called the *solvent front.*
   e. When the solvent front reaches the finish line, the chromatogram is finished. Open the chamber and remove the vial. Replace the lid.

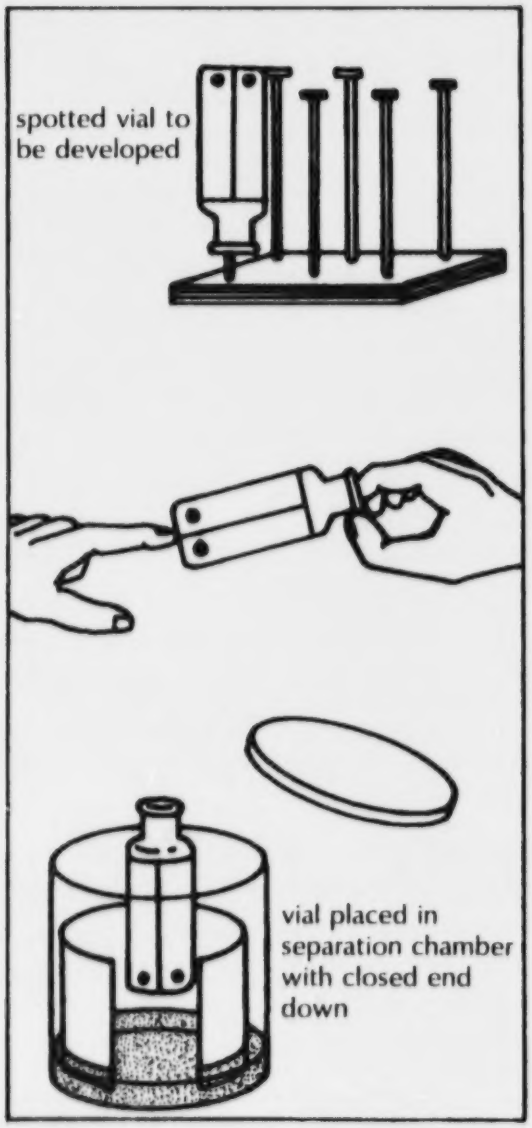


Figure 26 Placing thin-layer chromatogram vials in separation chambers

f. Make a data table similar to Table 2. On the table carefully draw the colors of the pigments in crayon, using a metric ruler if necessary, to show exactly where the bands are located on the chromatogram.

   Record the data immediately because the colors fade in a few hours.

2. Develop the plant pigment extract chromatograms.

   CAUTION: Petroleum ether and ethyl alcohol are both poisonous, flammable liquids. **Do not use them near any flame. Wear an apron and goggles.**

   a. Use a separation chamber containing a 9:1 mixture of petroleum ether and ethyl alcohol.

   b. Repeat the above procedure for developing thin-layer chromatograms.

Table 2 Sample Data Table for Thin-Layer Chromatograms

Sample _____

Age of sample _____

Sorbent _____

Solvent _____

Sketch the chromatogram here. Indicate **exact** distances between bands.

Description of separated substances by color	Identification of substances

Comments

Name of researcher _____

Date _____

Special Problems

Students who like to do chromatography or enjoy working with plant pigments may wish to investigate some of the following problems.

CAUTION: Be sure to have your teacher check and approve your experiment **before** you proceed.

1. What difference, if any, is there between pigments in marine algae (seaweed) and those in land plants?
2. What other substances can be used as sorbent materials?

   Try some sorbents other than paper and silica gel. A stick of soft chalk makes a chromatographic column. Powdered sugar is a chromatographic sorbent material. Each kind of sorbent and each type of sample requires its own solvent. Consult references in the back of this book for a list of solvents to use with chalk or sugar or other sorbents.
3. What types and colors of pigments can be found in such parts of plants as roots, stems, flowers, and fruits?
4. Do the same kinds of pigments occur in all plants? Consider the colors you see in crotons, coleus, and some of the colorful hedge plants.
5. What other methods can be used to separate plant pigments?

   Another interesting way to separate some kinds of plant pigments is a chemical method called *selective solubility*. Plant pigments can be roughly divided into two chief types, based on their solubility.

   a. *Sap pigments.* These are water-soluble. They occur in the sap or juice of any part of the plant. They can be extracted by boiling plant parts in water. For example, the water in which beets are boiled becomes red because of a sap pigment called *anthocyanin.*

b. *Plastid pigments.* These pigments, including the chlorophylls, are in tiny structures called *plastids* inside plant cells. Plastid pigments are not water-soluble but can be extracted with ethyl acetate, alcohol, or acetone.

Both sap pigments and plastid pigments can be extracted at the same time from plants by solvents that will dissolve both, such as boiling alcohol and acetone.

Materials

- mortar and pestle
- large vial
- 15 ml acetone
- 10 ml petroleum ether
- 2 small vials
- glass stirring rod
- pipette or syringe

CAUTION: Acetone and petroleum ether are poisonous, flammable liquids. **Do not use these liquids near any flame. Wear an apron and goggles.**

Directions

1. Obtain the parts of the plant you wish to extract (for example, a piece of root, some soft stems, and flower petals). Break them or cut them into small pieces, put them in the mortar, and grind with about 15 ml of acetone. Both the sap pigments and the plastid pigments will be extracted by the acetone.
2. Pour the acetone into a large vial. Add 10 ml of petroleum ether. Add 25 ml of water. Swirl or stir the mixture with a glass rod. Allow the layers to separate.
3. With a syringe, draw off each layer into a separate vial. Mark the vials. The sap pigments will be in the water layer. The plastid pigments will be in the petroleum ether layer.
4. Record the results in Table 3.

Table 3 Plant Pigments Found by Selective Solubility

Sap or plastid (S or P)	Dissolved in	Color	Part of plant it came from	Name or type of plant it came from

Glossary of Chromatographic Terms

Absorb. To take into, as water is taken into a sponge.

Adsorb. To adhere to the surface; to collect in condensed form on the surface of.

Capillary pipette. A glass or plastic tube with a very small opening used to transfer or measure small amounts of liquids.

Chromatography. An analytical method for separating substances in a complex mixture, particularly closely related substances or minute quantities difficult to separate by other chemical means.

Chromatogram. The visual result of chromatography showing the separated substances. Also called *chromatograph.*

Chromatographic paper. An even-textured, absorbent paper used in paper chromatography.

Origin. The position on a chromatogram where spots are originally placed.

PC. Paper chromatography.

Sample. Minute amount of material, usually dissolved in a solvent, to be separated by chromatography.

Scoring. The technique of scratching lines in the surface of a thin-layer chromatogram to mark the finish line or to separate samples.

Separation chamber. An airtight jar, test tube, or other container holding the solvent used in the development of a chromatogram.

Silica gel g. A very light powder used as the sorbent layer in thin-layer chromatography. The *g* indicates that a binder, calcium sulfate

(plaster of Paris), has been added to the gel to help make it stick to the glass vial.

Slurry. A viscous solution of liquid and a solid. In thin-layer chromatography, the slurry is made from water and silica gel.

Solvent. A liquid or a mixture of liquids used to separate substances in chromatography. Also referred to as the *solvent system.*

Solvent front. The leading edge of a moving solvent in a developing chromatogram.

Sorbent. The material or substance that absorbs or adsorbs substances in a mixture.

Spot. A small, concentrated deposit of a sample on a chromatogram; also, to put the sample on the chromatogram.

TLC. Thin-layer chromatography.

REFERENCES

Anwar, M. H. 1963. Separation of plant pigments by thin-layer chromatography. *J. Chem. Ed.* 40(1):29-30.

Baker, C. G. 1956. Chromatography and paper electrophoresis. *The Science Teacher* 23(1):192-195.

Beeskow, H. C. 1965. Color in plants. *School Science and Math* 65:545-552.

Biological Science Curriculum Study. 1970. Chromatography of chloroplast pigments. *BSCS Newsletter* 41(7).

Brinkman Instrument Co. 1971. *Introduction to thin-layer chromatography.* Brinkman Technical Bulletin T-54. Westbury, New York.

Heftman, E. 1967. *Chromatography.* Reinhold, New York.

Ikan, R., and E. Rapaport. 1962. Test tube and glass rod TLC. *J. Chem. Ed.* 44(5):297-298.

Jordan, D. 1968. Cylindrical TLC. *J. Chem. Ed.* 45:510.

Kraus, D. 1963. Rapid thin-layer chromatography of chloroplast pigments for the high school biology laboratory. *American Biology Teacher* 25(8):616-620.

Lauren, P. 1966. Chalk chromotography: three-dimensional separation. *The Science Teacher* 33(1):56-57.

Lederer, E., and M. Lederer. 1953. *Chromatography.* Elsevier, Amsterdam.

Stahl, E. 1965. *Thin-layer chromatography.* Springer-Verlag, New York.

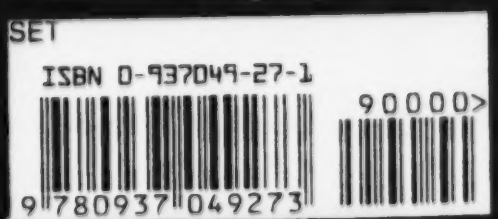
Tswett, M. 1967. Absorption analysis and chromatographic methods: application to the chemistry of chlorophylls. *J. Chem. Ed.* 44:238-242.

Curriculum Research & Development Group
University of Hawai'i
1776 University Avenue
Honolulu, Hawai'i 96822

Phone: 800 799-8111 or 808 956-4969
Fax: 808 956-6730
E-mail: crdg@hawaii.edu
www.hawaii.edu/crdg/

ISBN 0-937049-28-X

SET
ISBN 0-937049-27-1



BEST COPY AVAILABLE

U.S. Department of Education
Office of Educational Research and Improvement (OERI)
National Library of Education (NLE)
Educational Resources Information Center (ERIC)

NOTICE

Reproduction Basis

☑ This document is covered by a signed "Reproduction Release (Blanket)" form (on file within the ERIC system), encompassing all or classes of documents from its source organization and, therefore, does not require a "Specific Document" Release form.

☐ This document is Federally-funded, or carries its own permission to reproduce, or is otherwise in the public domain and, therefore, may be reproduced by ERIC without a signed Reproduction Release form (either "Specific Document" or "Blanket").